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SHORT NOTE

Life history traits of adults and embryos of the Antarctic midge *Belgica antarctica*

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Abstract Although larvae of the Antarctic midge, *Belgica antarctica*, live for more than 2 years, the adult and embryonic stages are brief and are less well known than the larvae. In this report, we provide additional details of these understudied life stages with laboratory observation on adult emergence, longevity, preoviposition period and embryonic development. Male adults emerged slightly earlier than females, and they also lived longer. More than a half (57 %) of the adults that emerged in the laboratory were males. Females produced only a single egg mass and died within a day after oviposition. Embryonic development required 16 days at 4 °C, and prior to hatching, the pharate larvae perform a distinct sequence of behaviors that include drinking and peristaltic movement. We also discuss points that need to be resolved for laboratory propagation of this species.

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Introduction

The midge, *Belgica antarctica*, is the southernmost insect and is the largest permanent free-living terrestrial animal in Antarctica. It is the only insect endemic to Antarctica, and only one other species (*Belgica albipes*), a resident of sub-Antarctic islands, belongs to the same genus (Convey and Block 1996). *B. antarctica* is restricted to the west coast of the Antarctic Peninsula and the South Shetland Islands; although it is locally abundant, it has a patchy distribution (Sugg et al. 1983). The midge is most commonly observed in moderately damp sites, especially those enriched by vertebrate feces (Strong 1967; Edwards and Baust 1981). Particularly dense aggregations of larvae have been found associated with the nitrophilous alga *Prasiola crispa*. Larvae are considered to be non-selective feeders (Baust and Edwards 1979) that are known to eat dead plant material, algae and microorganisms including fungi (Strong 1967; Edwards and Baust 1981). The wingless adults, approximately 3 mm in length, emerge during the summer after a brief pupal period and mate within aggregations. Many details of the life cycle of *B. antarctica* have been described, especially features of the 2-year period of larval development (Usher and Edwards 1984).

The unique features of this extremophile stimulated a number of detailed field observations a few decades ago, but only recently has it been possible to investigate physiological and molecular mechanisms of their cold tolerance (e.g., Lee et al. 2006; Rinehart et al. 2006; Benoit et al. 2007; Teets et al. 2012; Kawarasaki et al. 2013). However, all previous studies have completely depended on using

field-collected animals because a method for maintaining a laboratory colony has not been established. Thus, many details of the life history of *B. antarctica* remain fragmentary and are strictly based on field observations. In the present study, we observed spontaneous adult emergence possibly induced by artificial summer conditions and also monitored sex ratio, longevity, reproductive output, embryogenesis and hatching behavior of this midge in the laboratory, with the expectation that some of this information may prove helpful in developing techniques for propagating this species in the laboratory.

Materials and methods

Larvae of *B. antarctica* were collected on several islands near Palmer Station, Antarctica (64°46′S, 64°04′W) in January 2012. They were transported in a substrate consisting of detritus, moss and algae to Ohio State University and thereafter to Osaka City University (Permission No. 24K134, Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries Japan). At Osaka City University, larvae were maintained with the substrate in airtight steel boxes (size: 20 × 14.5 × 5.5 cm). All observations were done under an 18 h light and 6 h dark cycle (LD = 18:6 h) at 4 °C, conditions that approximated their summer habitat. The substrate in the boxes was sprayed with water once every 2 days to maintain high humidity.

Adults were transferred on the day of emergence to an airtight plastic container (diameter 10 cm, height 5.3 cm) provisioned with a wet paper towel and maintained at LD 18:6 h at 4 °C. The paper towels were sprayed with water daily to retain a high humidity. Adults were observed daily to verify longevity and egg production.

Newly laid egg masses were transferred to a plastic dish (diameter 5.0 cm, height 2.0 cm) with a piece of moist paper. Dishes were sealed with Parafilm (Pechiney Plastic Packaging Company, Chicago) to maintain high moisture levels and were maintained at 4 °C, at LD 18:6 h. Eggs were observed daily under a stereoscopic microscope (SMZ1500; Nikon, Tokyo) and, even during microscopic observations, were kept at 4–7 °C using crushed ice.

Results

Spontaneous adult emergence was observed in the laboratory at 4 °C in June and July 2012, approximately 6 months after larvae were collected in the field (Fig. 1a). Over a period of approximately 1 month, we observed emergence of 120 adult males and 88 females, indicating a significant bias in the sex ratio of the adults that emerged (χ^2 test, $p < 0.05$). Although significant protandry was detected in adult emergence (Fig. 1; Mann–Whitney *U* test, $p < 0.05$), this difference was not highly pronounced.

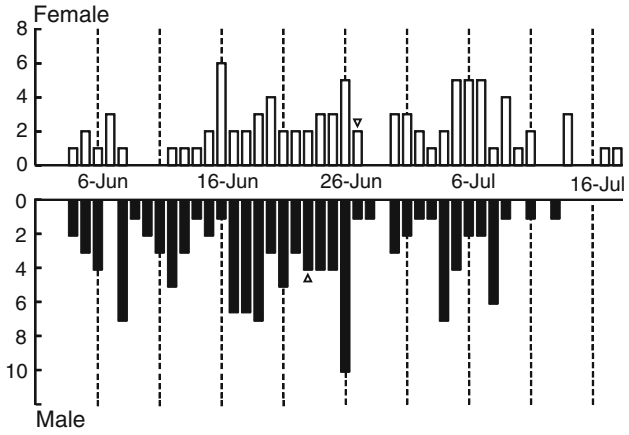

Fig. 1 Sex of adults of *B. antarctica* that emerged from June 4 to July 18, 2012, in the laboratory

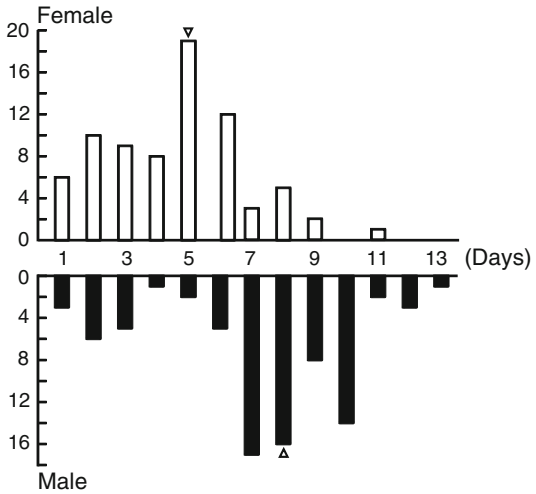

Fig. 2 Longevity of male and females adults of *B. antarctica*

Table 1 The number of egg masses laid by *B. antarctica* females

No. of egg masses per female	No. of females
2	2 (2.3 %)
1	49 (57.0 %)
0	35 (40.7 %)
Sum	86 (100 %)

Adult males lived significantly longer (median of 8 days, $n = 83$) than females (median of 5 days, $n = 76$) (Fig. 2; Mann–Whitney *U* test, $p < 0.05$). These results generally agree with the data by Edwards and Baust (1981). In the present study, more than half the females (59.3 %)

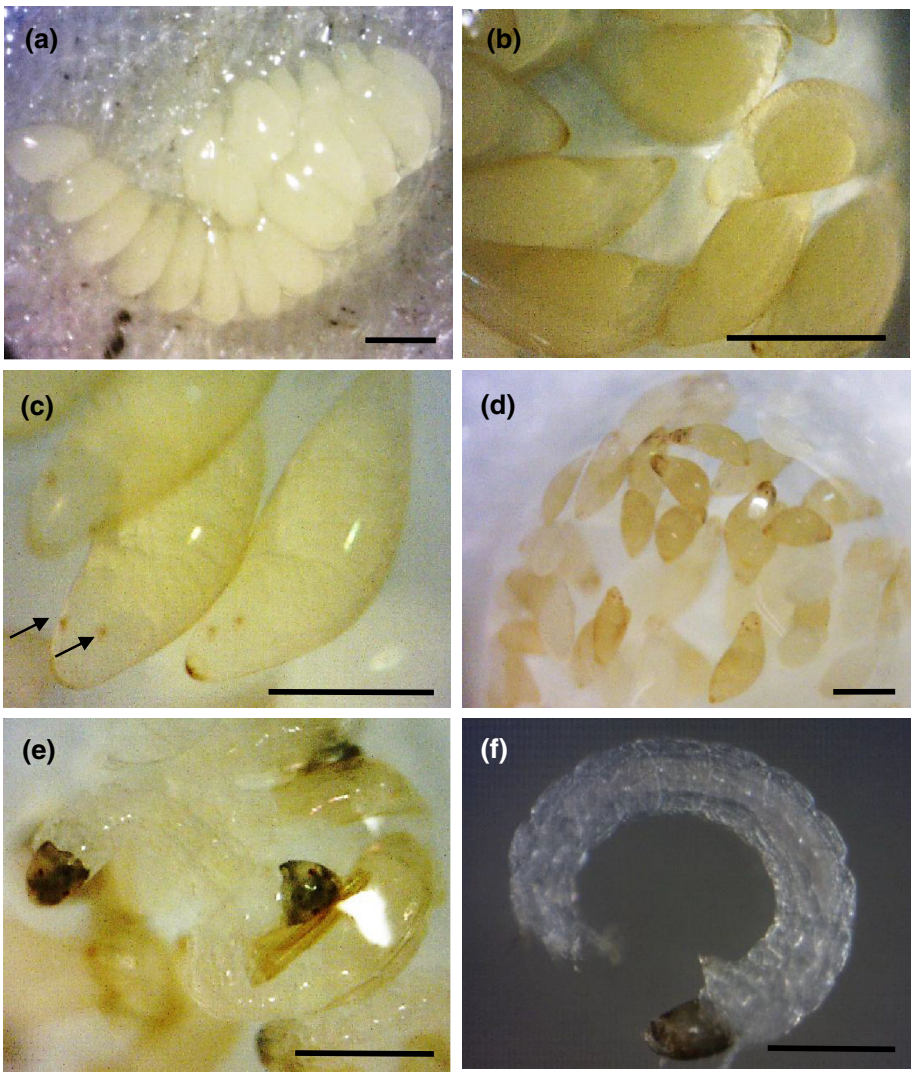

Fig. 3 Eggs and embryos of *B. antarctica*. An egg mass just after oviposition (day 0, **a**). Eggs that were fertilized gradually turned yellow within a few days (day 7, **b**). Eyes (*arrows*) appeared 10 days after oviposition (**c**). Pharate larvae just before hatching (day 15, **d**; day 16, **e**). First instar larva (2 days after hatching, **f**). *Scale bars* 300 μm

laid egg masses (Table 1). Eggs were deposited in a hygroscopic gelatinous mass, and most masses were coiled and ribbon-shaped (Fig. 3a). Most females produced only a single egg mass (Table 1), although 2 females produced 2 egg masses. The median preoviposition period was 3 days (interquartile range was 2–5 days; $n = 51$). Females died within a day after oviposition or during oviposition, except for the two individuals that reproduced twice.

We collected 55 egg masses. However, embryonic development was observed in only 8 of the masses (15.1 %); no signs of development were observed in the others. The percentage of fertilized eggs in an egg batch that contained some fertilized eggs was 28.1 % ($n = 368$). We also observed that virgin females were able to lay unfertilized eggs ($n = 2$). A single egg batch contained a median of 41 eggs (interquartile range was 29–53 eggs; $n = 50$). Immediately after oviposition, the eggs were whitish and opaque (Fig. 3a), but if they were fertilized, the eggs gradually became yellowish and transparent and the yolk was concentrated at the center of the eggs about 7 days after oviposition (Fig. 3b). The larval eyes were apparent 10 days after oviposition (Fig. 3c), and then, the entire head became pigmented and the eyes progressively changed from red to dark red (Fig. 3d). Hatching occurred 16 days after oviposition (Fig. 3e). For a subset of eggs, we were not able to control the temperature conditions precisely because of failure of an environmental chamber, and therefore, the temperature in the chamber became variable (4–13 °C). In those cases, hatching occurred within 10 days after oviposition. During development, embryos increased in size and eventually filled the entire space within the chorion. Pharate larvae within the chorion were eventually U-shaped and spirally twisted. At least 1 day before hatching, the pharate larvae started "drinking behavior" (Online Resource 1) to increase the internal pressure to rupture the chorion (Davis 1966), and thereafter, they demonstrated peristaltic movement of their bodies. Extensive movement of the pharate larvae within the chorion induced a sudden rupture of the

chorion, and subsequent twisting movements resulted in the larva becoming completely free of the chorion within a few minutes (Online Resource 2). The hatched larvae were transparent for a few days (Fig. 3f) but gradually became pigmented thereafter.

Discussion

Temperature effects on adult emergence and egg hatching

In the laboratory, we observed spontaneous adult emergence in June and July, emergence that was approximately 6 months earlier than the late December–early January emergence observed in the field. This suggests that larvae in the field are competent to proceed with development much earlier than late December, but the prevailing low temperatures of winter in Antarctica likely prevent this from occurring until favorable temperatures return. Indeed, early warming in the spring of 2006 produced early adult emergence of *B. antarctica* in early December and possibly even in November (Schulte et al. 2008). The synchronized emergence behavior of high latitude (Armitage et al. 1995) and Arctic (Danks and Oliver 1972) chironomids is controlled by a heat-sum threshold. Thus, adult emergence of chironomids inhabiting high latitudes would be largely dependent on a heat sum. It is largely unknown whether *B. antarctica* can respond to photoperiod (photoperiodism), but prolonged summer photoperiod in the laboratory may also affect the spontaneous adult emergence. We also noted that developmental rates are strongly influenced by temperature, as shown in other species: the duration of embryogenesis was 16 days at 4 °C, but it can be completed within 10 days at higher temperatures, ranging from 4 to 13 °C. It is well known that the Antarctic Peninsula has undergone rapid climatic warming recently (Vaughan et al. 2003; Ding et al. 2011). This is especially obvious at Palmer Station, where a steady, consistent rise in average daily temperature of ~0.1 °C has been reported every 2 years over a short period of 20 years from 1990 to 2010 (Teets and Denlinger 2014). Such warming would directly promote early adult emergence, as well as early hatching of *B. antarctica*. Larvae may have lasting benefits of a prolonged summer to grow and molt. However, it is still uncertain how this warming affects the life cycle of *B. antarctica*. An understanding of the physiological plasticity of *B. antarctica* to environmental perturbations is necessary to forecast the future dynamics of this species.

Points that need to be resolved for laboratory propagation

There is conflicting evidence on the sex ratio of *B. antarctica*. Several papers report a strong male sex bias: 82 % males reported by Wirth and Gressitt (1967) and 80 % males reported by Strong (1967). Edwards and Baust (1981) collected adults on the ground surface and found that male predominance persisted throughout the summer; the mean ratio in surface aggregations (austral summer 1977–1978) and from sticky traps (1978–1979) was nearly 6:1 (male/female), with a steady seasonal decline from 10–20:1 to 2–5:1 (Edwards and Baust 1981). By contrast, Peckham (1971) reported an almost equal ratio (55 % males) in adults collected on January 1966 and suggested that the bias reported for males may be correlated with sexual differences in behavior or sampling techniques. Based on the sex of pupal exuviae examined, Edwards and Baust (1981) assumed that there was no sex bias at the time of emergence. They suggested that the observed sex bias in field observations was derived from differences in microhabitat selection between adult males and females. Males prefer to walk on the ground surface, but females tend to remain in subsurface cavities. The sex ratio obtained from subsurface cavities more closely approximated that found by examining pupal exuviae (Edwards and Baust 1981). In the present study, we detected a significant bias in the sex ratio at the time of adult emergence in the laboratory. However, the ratio was not greatly skewed (57 % adults were males), and it was similar to the data from Peckham (1971). Thus, we assume that there is no bias in the sex ratio of this species.

Protandry, the situation where males emerge before females, was postulated in *Belgica* by Sugg et al. (1983), based on the field-collected pupae and pupal exuviae. They also noted evident protandry at the time of pupation. In their observations, males pupated approximately 4 days earlier than females. The present study directly verified protandry at the time of adult emergence. Protandry is common, although not universal, among the chironomids (Armitage et al. 1995). The Australian chironomid *Chironomus tepperi* shows clear protandry, with males developing significantly faster than females across a range of temperatures (Stevens 1998). Danks and Oliver (1972) also reported protandry in several Arctic chironomids. Protandry is especially advantageous for species with short-lived adults; an abundance of ready-to-mate males awaiting female emergence or arrival increases the chances of mating success (Morbey and Ydenberg 2001).

In the present study, only a few eggs hatched. Only a few of the egg masses contained fertile eggs, and usually only a few eggs within such egg masses were fertile. To establish laboratory colonies, the low fertility rate must be improved. Swarming behavior is known to be extremely important for successful reproduction in temperate chironomids (Armitage et al. 1995). For example, in *Chironomus riparius*, reduced swarming in a series of swarming suppression cages resulted in fewer matings and less

oviposition, and with total suppression of swarming, no mating occurred (Caspary and Downe 1971). In *Belgica*, Edwards and Baust (1981) suggested that the predominance of adult males on the ground surface corresponds to the male swarming behavior of winged chironomids. Protandry would also foster male predominance in the mating sites. However, in the present study, we unintentionally prevented swarming: adults that emerged on the same day were placed in a single container to monitor longevity. Thus, our experimental setup did not allow for the advantages of protandry. In addition, the number of adults in a single container was small (in most cases, 3–10 individuals), because the number of adults emerging in a single day was limited. Furthermore, the sex ratio was not biased toward males in this procedure (in most cases male/female = 0.2–2:1). Strong (1967) reported that female adults need to copulate immediately after emergence; otherwise, they produce unfertilized eggs. Thus, preventing swarming behavior, as in this study, may severely reduce fecundity. Synchronous adult emergence is thus likely to be essential for swarming and consequently high fecundity. To establish a laboratory colony, it will thus be essential to develop a protocol for synchronous adult emergence, and the key to generating synchronous emergence will likely be the mimicking of the natural thermal conditions that serve to synchronize their life cycle under field conditions.

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References

Armitage PD, Pinder LC, Cranston PS (1995) The Chironomidae: biology and ecology of non-biting midges. Chapman & Hall, London

Baust J, Edwards JS (1979) Mechanisms of freezing tolerance in an Antarctic midge *Belgica antarctica*. Physiol Entomol 4:1–5

Benoit JB, Lopez-Martinez G, Michaud MR, Elnitsky MA, Lee RE Jr, Denlinger DL (2007) Mechanisms to reduce dehydration stress in larvae of the Antarctic midge, *Belgica antarctica*. J Insect Physiol 53:656–667

Caspary VG, Downe AER (1971) Swarming and mating of *Chironomus riparius* (Diptera: Chironomidae). Can Entomol 103:444–448

Convey P, Block W (1996) Antarctic Diptera: ecology, physiology and distribution. Eur J Entomol 93:1–13

Danks HV, Oliver DR (1972) Seasonal emergence of some high arctic Chironomidae (Diptera). Can Entomol 104:661–686

Davis CC (1966) A study of the hatching process in aquatic invertebrates. XVI. Events of eclosion in *Calopsectra neoflavellus* Malloch (Diptera, Tendipedidae). XVII. Hatching in *Argulus megalops* Smith (Crustacea, Branchiura). Hydrobiologia 27:196–207

Ding Q, Steig EJ, Battisti DS, Küttel M (2011) Winter warming in West Antarctica caused by central tropical Pacific warming. Nat Geosci 4:398–403

Edwards JS, Baust J (1981) Sex ratio and adult behavior of the Antarctic midge *Belgica antarctica* (Diptera, Chironomidae). Ecol Entomol 6:239–243

Kawarasaki Y, Teets NM, Denlinger DL, Lee RE Jr (2013) The protective effect of rapid cold-hardening develops more quickly in frozen versus supercooled larvae of the Antarctic midge, *Belgica antarctica*. J Exp Biol 2016:3937–3945

Lee RE Jr, Elnitsky MA, Rinehart JP, Hayward SA, Sandro LH, Denlinger DL (2006) Rapid cold-hardening increases the freezing tolerance of the Antarctic midge *Belgica antarctica*. J Exp Biol 209:399–406

Morbey YE, Ydenberg RC (2001) Protandrous arrival timing to breeding areas. Ecol Lett 4:663–667

Peckham V (1971) Notes on the chironomid midge *Belgica antarctica* Jacobs at Anvers Island in the maritime Antarctic. Pac Insects Monogr 25:145–166

Rinehart JP, Hayward SA, Elnitsky MA, Sandro LH, Lee RE Jr, Denlinger DL (2006) Continuous up-regulation of heat shock proteins in larvae, but not adults of a polar insect. Proc Natl Acad Sci USA 103:14223–14227

Schulte GG, Elnitsky MA, Benoit JB, Denlinger DL, Lee RE Jr (2008) Extremely large aggregations of collembolan eggs on Humble Island, Antarctica: a response to early seasonal warming? Polar Biol 31:889–892

Stevens MM (1998) Development and survival of *Chironomus tepperi* Skuse (Diptera: Chironomidae) at a range of constant temperatures. Aquat Insects 20:181–188

Strong J (1967) Ecology of terrestrial arthropods at Palmer Station, Antarctic Peninsula. Antarct Res Ser Natl Res Counc 10:357–371

Sugg P, Edwards JS, Baust J (1983) Phenology and life history of *Belgica antarctica*, an Antarctic midge (Diptera: Chironomidae). Ecol Entomol 8:105–113

Teets NM, Denlinger DL (2014) Surviving in a frozen desert: environmental stress physiology of terrestrial Antarctic arthropods. J Exp Biol 217:84–93

Teets NM, Peyton JT, Colinet H, Renault D, Kelley JL, Kawarasaki Y, Lee RE Jr, Denlinger DL (2012) Gene expression changes governing extreme dehydration tolerance in an Antarctic insect. Proc Natl Acad Sci USA 109:20744–20749

Usher MB, Edwards M (1984) A dipteran from south of the Antarctic Circle: *Belgica antarctica* (Chironomidae) with a description of its larva. Biol J Linn Soc 23:19–31

Vaughan DG, Marshall GJ, Connolley WM, Parkinson C, Mulvaney R, Hodgson DA, King JC, Pudsey CJ, Turner J (2003) Recent rapid regional climate warming on the Antarctic Peninsula. Clim Change 60:243–274

Wirth WW, Gressitt JL (1967) Diptera: Chironomidae (midges). Antarct Res Ser Natl Res Counc 10:197–203